紅龍果濕腐病之新紀錄病原菌 *Gilbertella persicaria* var. *pitaya* 及其防治

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摘 要

紅龍果 (Hylocereus spp.) 為台灣重要新興作物之一，田間感染濕腐病之花苞、花瓣及幼果出現褐黑色凹陷、水浸狀潰爛，或是幼果發育不良，尤其紅肉果實容易出現黑心等病徵；感染濕腐病的成熟果實，病斑呈現水浸狀軟腐，自表皮或鱗片迅速擴展，3-5 天內感染整顆果實。藉由形態特徵與 ITS DNA 序列比對結果，確認引起紅龍果果實濕腐病的病原菌近似於 *Gilbertella persicaria*，為台灣新紀錄病原菌。然而台灣分離到之分離株孢囊壁型態、病原性，以及 ITS 序列相較標準株 (holotype，自水蜜桃果實分離得)具些微差異，故另稱之 *G. persicaria* var. *pitaya*。人工接種紅龍果成熟果實結果顯示，*G. persicaria* var. *pitaya* 多數分離株需經傷口才能成功感染果實。在測試可能之寄主範圍試驗顯示，*G. persicaria* var. *pitaya* 可感染 (1) 無論有無傷痍處理的水蜜桃與番石榴，但在番石榴上發病率低；(2) 有傷痍處理之硬柿、梨子、李子、蘋果、芒果、蓮霧、及番茄；(3) 楊桃、奇異果、美濃香瓜與香蕉則沒有明顯病徵。為篩選有效的防治藥劑，在 PDA 培養基中測試不同藥劑抑制效果。化學合成藥劑抑制菌絲生長效果：以賽普護汰寧效果最佳，百克敏、撲克拉錳、三得芬、待克利、得克利以及克熱淨次之；化學合成藥劑抑制孢囊孢子發芽效果：以扶吉胺效果最佳，待克利與賽普護汰寧次之，三得芬、免得爛、得克利以及撲克拉錳再次之。其中，賽普護汰寧、百克敏、得克利、克熱淨以及免得爛為植保手冊紅龍果推薦用藥，可立即應用於田間防治病害；非化學合成藥劑抑制菌絲生長效果中，黑修羅與炭無蹤等肉桂油微乳劑，及 2-2 與 4-4 式波爾多液皆有良好抑制效果；非化學合成藥劑抑制孢囊孢子發芽效果中，黑修羅與炭無蹤等肉桂油微乳劑、2-2 與 4-4 式波爾多液，以及亞磷酸等，亦有良好抑制效果佳。根據田間生態調查，濕腐病好發於下雨過後，建議下雨前可使用抑制孢子發芽之藥劑保護幼嫩花瓣與幼果，下雨後使用抑制菌絲生長之藥劑治療罹病組織，並應盡速拔除罹病花並帶離果園，避免病菌往子房部位感染，造成果實發育不良出現黑心病徵，且建議勿於下雨後採收果實。病菌容易由果實切口處侵入，摘果實時可預留長果梗，以防病菌由切口感染後快速入侵果實。

Introduction

Pitaya (*Hylocereus* spp.) is one of the important newly emerging fruit crops in Taiwan. However, the fruit production is limited by pitaya canker caused by *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers and wet rot caused by *Gilbertella persicaria* (Eddy) Hesseltine, which were found in 2009 and 2012 respectively. This paper would focus on the disease of wet rot, and the pathogen identification and management of the diseases would be introduced in.

Symptoms of pitaya wet rot

Pitaya wet rot severely affected flowering, fruiting and postharvest storage of pitaya in Taiwan. In the disease survey of pitaya orchards, the infection occurred mainly during rainy season. Diseased flower buds (Fig. 1A), petals (Fig. 1B) and young fruits (Fig. 1C) showed brown and small water-soaked lesion. Most of the infected fruits in the orchard failed to develop normally and dropped prematurely. Internal black rot of fruits could also be observed when the infection occurred during fruiting (Fig. 1D). Mature fruits were infected when being harvested or during storage and appeared water-soaked and soft rot on the fruit appearance (Fig. 1E). Wet rot of fruits usually initiated from the stem-ends, occasionally from the middle of fruits or scales, and could develop into completely fruit rot 3-5 days after infection. After harvest, fruits could be infected through the wounds and become rotted during storage or transportation. Brown to black sporangia of *G. persicaria* var. *pitaya* could be observed producing on the surface of infected tissues, especially under humid conditions.

Identification and differences of *Gilbertella persicaria* var. *pitaya*

According to the description by Benny (1991), the taxonomic classification of *G. persicaria* is as follows:

- Fungi
  - Zygomycota
    - Zygomycetes
      - Mucorales
        - Gilbertellaceae

The morphology of *G. persicaria* var. *pitaya* (Fig. 2) was similar with the description of the holotype of *G. persicaria* (CBS 190.32, isolated from rot peach, *G. persicaria* var. *persicaria*) (table 1). However, interestingly, in *G. persicaria* var.

pitaya, the number of longitudinal sutures on the sporangial wall were found no less than 1 and splitting the sporangial wall into 2-7, usually 4, equal pieces, while there was only 1 suture in the holotype; the number of the appendages at the ends of the sporangiospores of the pitaya isolates were 0-3 while 2-7 in the holotype.

In addition to the morphological characteristics, identity of *G. persicaria* var. *pitaya* was also confirmed by ITS (ITS1-5.8S-ITS2) sequence analysis. The identity between *G. persicaria* var. *pitaya* and the holotype of *G. persicaria* was 98.4%, with 5 additional and 6 different nucleotides in the *G. persicaria* var. *pitaya* isolates.

We also inoculated fruits of pitaya with the *G. persicaira* var. *persicaria* (CBS190.32, the holotype, and F209130). The water-soak lesions caused by these isolates were smaller and browner than those of *G. persicaria* var. *pitaya* caused.

In summary, these data suggested the *G. persicaria* var. *pitaya* was different from the holotype of *G. persicaria* in regards of the sporangial wall morphology, ITS sequence and virulence to fruits of pitaya.

Occasionally, field survey also showed that *Rhizopus stolonifer* could be found in the petal of withered flowers and might be confused with *G. persicaria* var. *pitaya*. These fungi can be distinguished by morphology and growth maximum temperature. The differences are listed in the table 2.

Pathogenicity of *Gilbertella persicaria* var. *pitaya*

The optimal temperatures for *G. persicaria* mycelial growth were 24-36 ^{O}C (Fig. 3), maximum at 38 ^{o}C. In order to understand the effect of wounding on the disease development of pitaya wet rot, healthy mature fruits were collected from the field for artificial inoculation with sporangiospores of the pathogen. The tests results showed that wounding resulted in severe infection (table 3).

Results of artificial inoculations also showed that *G. persicaira* var. *pitaya* were pathogenic on wounded and unwounded fruits of peach and guava. The pathogen was also pathogenic on wounded fruits of apple, mango, persimmon, plum and tomato, and was non-pathogenic on banana, kiwifruit, starfruit and melon (table 4). These results suggested that *G. persicaira* var. *pitaya* might be a potential pathogen for these sensitive fruits in the field or upon marketing and affect the storage periods in the market.

Chemicals selection for the control of wet rot

Synthetic chemicals with different inhibition mechanism were selected to test the inhibition to *G. persicaira* var. *pitaya* on agar media, although some chemicals, like

fluazinam, difenoconazole, tridemorph and prochloraz-Mn have not been yet authorized to be used on pitaya. The data showed that the mycelial growth was completely inhibited by cyprodinil + fludinoxonil at 1 ppm a.i., and pyraclostrobin, prochloraz-Mn, tridemorph, difenoconazole and tebuconazole at 100 ppm a.i.. The sporangiospore germination was completely inhibited by fluazinam at 1 ppm a.i., and cyprodinil + fludinoxonil, difenoconazole, metiram, tebuconazole, tridemorph, prochloraz-Mn and pyraclostrobin at 10 or 100 a.i..

The tests of effect of some control agents for organic farming on pathogen were also conducted on agar media. The data showed that neutralized phosphorous acid and lime sulfur solution could inhibit sporangiospores germination at 1000 and 500 dilution rate respectively while Bordeaux mixture with formulas of 2-2-1 and 4-4-1 (copper sulfate (g) - lime (g) - water (L)) could inhibit both mycelial growth and sporangiospores germination. As for the plant extracts, both cinnamon oil products - Hey-Show-Lo and Tan-Wu-Zung - could inhibit mycelial growth and sporangiospores germination at 1000 and 4000 dilution rate respectively, lemongrass oil product - Mei-Tsow - did at 1000 and 500 dilution rate respectively, and chinese gall extract product - Hwo-Li-Nen - did both at 500 dilution rate.

The efficiency of these agents for controlling wet rot for organic management in the field would be further tested.

Management suggestions of wet rot

According to the epidemic investigation, the disease severity of pitaya wet rot is closely related with the occurrence and frequency of the raining. In addition, the sporangia would emerge from the diseased tissue quickly under high humidity, and the sporangiospores could spread out by rain drops and wind.

Based on the epidemic and pathogenic studies so far, advices are given as follows.

1. To reduce the inoculum, flowers and fruits with wet rot symptoms should be excised, and then packed and removed out of the orchards immediately.
2. For crop protection in the flowering period in the fields, sporangiospores-germination-inhibiting chemicals and mycelia-growth-inhibiting chemicals are recommended to be used before and after raining respectively.
3. In order to reduce fruit infection, harvesting the fruits on the raining day should be avoided, and the wounds and the surface of the fruits should be dried before packaging.

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Pathogen Identification and Management of Flower and Fruit Wet Rot of pitaya (*Hylocereus* spp.) Caused by G*ilbertella persicaria* var. *pitaya*

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Abstract

Pitaya (*Hylocereus* spp.) is one of the important newly emerging fruit crops in Taiwan. Fruit wet rot, a severe postharvest disease of pitaya caused by *Gilbertella persicaria* var. *pitaya*, was first found in August 2009. Fruit infection occurred in the field during rainy season with a small water-soaked lesion around the stem-end, which developed into fruit soft rot within 3-5 days after harvest. Occasionally, fruit soft rot initiated from lesions on fruit skin or scales, and internal black rot of fruit were also observed. Results of survey of pitaya orchards showed that infection of *G. persicaria* var. *pitaya* on young fruits or on flower buds and petals resulted in the development of symptoms similar to that observed in the postharvest fruits. Most of the infected fruits in the orchard failed to develop normally and dropped prematurely. However, in regards of the differences of the number of splitted sporangial wall, ITS sequence and virulence to fruits of pitaya, the *G. persicaria* var. *pitaya* was different from the holotype of *G. persicaria*. Brown to black sporangia of *G. persicaria* var. *pitaya* were formed on the surface of infected tissues, especially under humid conditions. Our data showed that tissue wounding was an important factor affecting the disease severity. *G. persicaira* var. *pitaya* were pathogenic on unwounded fruits of peach and guava, and wounded fruits of apple, mango, persimmon, plum, wax apple and tomato, but they were non-pathogenic on starfruit, melon, banana and kiwifruits. The results of chemical selection showed that cyprodinil + fludinoxonil, pyraclostrobin, prochloraz-Mn, tridemorph, difenoconazole and tebuconazole, fluazinam were the potential fungicides for controlling the disease. As for organic farming, the data showed that Bordeaux mixture and both cinnamon oil products, -Hey-Show-Lo and Tan-Wu-Zung- could inhibit mycelial growth and sporangiospores germination well. To control the disease, apply mycelial-growth- inhibiting chemicals the before raining to protect the fallowers and young fruits from infection and sporangiospores-germination- inhibiting chemicals after raining to treat the infected tissues. The infected tissues should be excised out of the orchards immediately therefore avoiding further infection of the fruiting fruits or other tissues. Also, harvest in the raining day should be avoided to reduce the infection rate during storage.

Table 1. Morphological characteristics of *Gilbertella persicaria* var. *persicaria* and *G. persicaria* var. *pitaya*.

Characters	*Gilbertella persicaria* var. *persicaria*	*Gilbertella persicaria* var. *pitaya*
Sporangiophore (branched or rarely branched; bent when young, upright at maturity)	19-33 µm in diam	18-50 µm in diam
Sporangia (yellow to dark brown, more or less globose; covered whith calcium oxalate crystals)	45-170 µm in diam.	60-115 µm in diam
Columella (with basal collar; obpyriform, obovoid to cylindrical)	36-81 µm in diam. at the widest	25-60 µm in diam. at the widest
No. of longitudinal suture (on the surface of sporangia wall)	1	1-5
No. of separated sporangial wall	2	2-7 (avg. 4)
Sporanigospores (globose, ellipsoid to ovoid)	5.1-17.8 × 3.8-12.7 µm	5.5-11.8 × 4.5-8.8 µm
No. of hyaline appendages (up to 24 µm, at the ends of sporanigospores)	2-7	0-3
Chlamydospores (globose to irregular; light brown, smooth-walled)	15-29 × 10-16 µm	18.9-25.4 × 14.9-23.8 µm

Table 2. Differences of diagnostic characters between *Rhizopus stolonifer* and *Gilbertalla persicaria* var. *pitaya*.

Characters	*Rhizopus stolonifer*	*Gilbertalla persicaria*
Sporangiospore with appendages	No	Yes
Max. growth temp.	33 ^{o}C	38 ^{o}C
Rhizoid	Yes	No
Sporangiophores	Branchless; erect	Branched or rarely branched; bent or upright
Sporangia diam.	Up to 200-300 µm	Less than 180 µm.
Sporangial wall	No	Yes

Table 3. Effect of wounded treatment on infection rates of pitaya fruit caused by *Gilbertalla persicaria* var. *pitaya* [1].

Isolate	dpi[2]	Infection rates (%)	
		Unwounded	Wounded
F212122	1	0	100
	2	77.7 (±29.8)	100
F210187	1	0	100
	2	0	100

1: The harvested fruits (*Hylocereus undatus*) were inoculated with cottons which were pre-immersed in spores suspensions for 1-3 min; wound were made by bug needles; spores suspensions concentration = 2× 10^6 spores/ml. This experiment were repeated for 2 times

2: dpi stands for “day post infection”.

Table 4. Test of fruit host ranges of *Gibertela persicaria* var. *pitaya* in Taiwan [1]

Name of tested fruit	Infection rates(%)[2]	
	Unwounded	Wounded
Peach (*Prunus persica*)	100	100
Guava (*Psidium guajava*)	16.7	33.3
Pear (*Pyrus pyrifolia*)	0	100
Plum (*Prunus salicina*)	0	100
Persimmon (*Diospyros kaki*)	0	100
Tomato (*Solanum lycopersicum*)	0	100
Mango (*Mangifera indica*)	0	91.7
Apple (*Malus domestica*)	0	87.5
Wax apple (*Syzygium samarangense*)	0	50
Banana (*Musa sapientum*)	0	0
Kiwifruit (*Actinidia deliciosa*)	0	0
Melon (*Cucumis melo*)	0	0
Starfruit (*Averrhoa carambola*)	0	0

1. Fruits were inoculated with cottons which were pre-immersed in spores suspensions of *G. persicaria* var. *pitaya* isolate F210187 for 1-3 min and infection was counted 72 hr post infection; wounds were made by bug needles; spores suspensions concentration = 2× 10^6 spores/ml. This experiment was repeated for 1-2 times.

2: The inoculation sites were counted as infected only if soft-rot symptoms appeared within 7 day post inoculation and *G. persicaria* var. *pitaya* can be reisolated again.

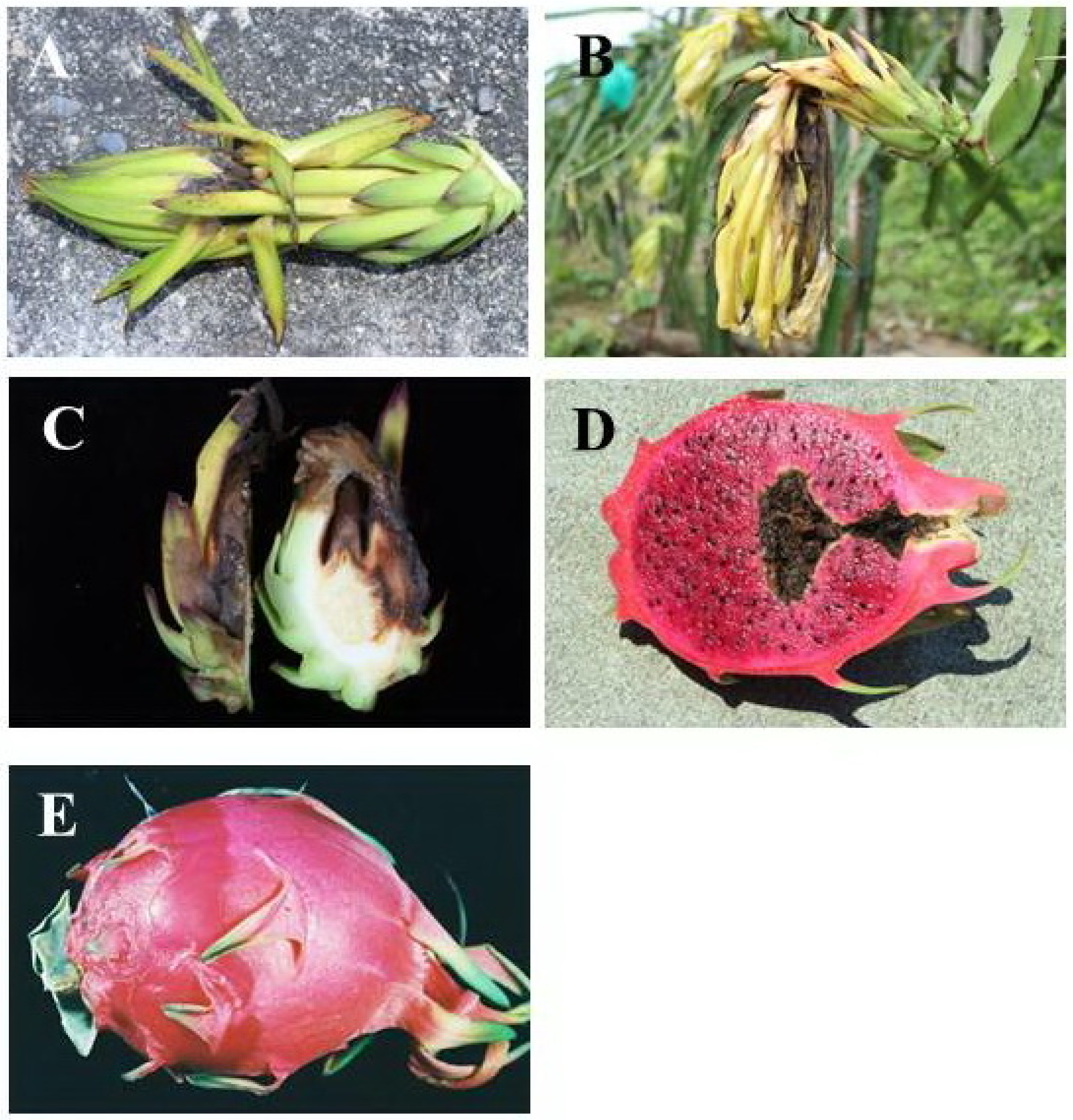

Fig. 1. *Gilbertella persicaria* caused wet rot symptoms on flower bud (A), petals (B), young fruit (C, D) and harvested fruit of pitaya (E).

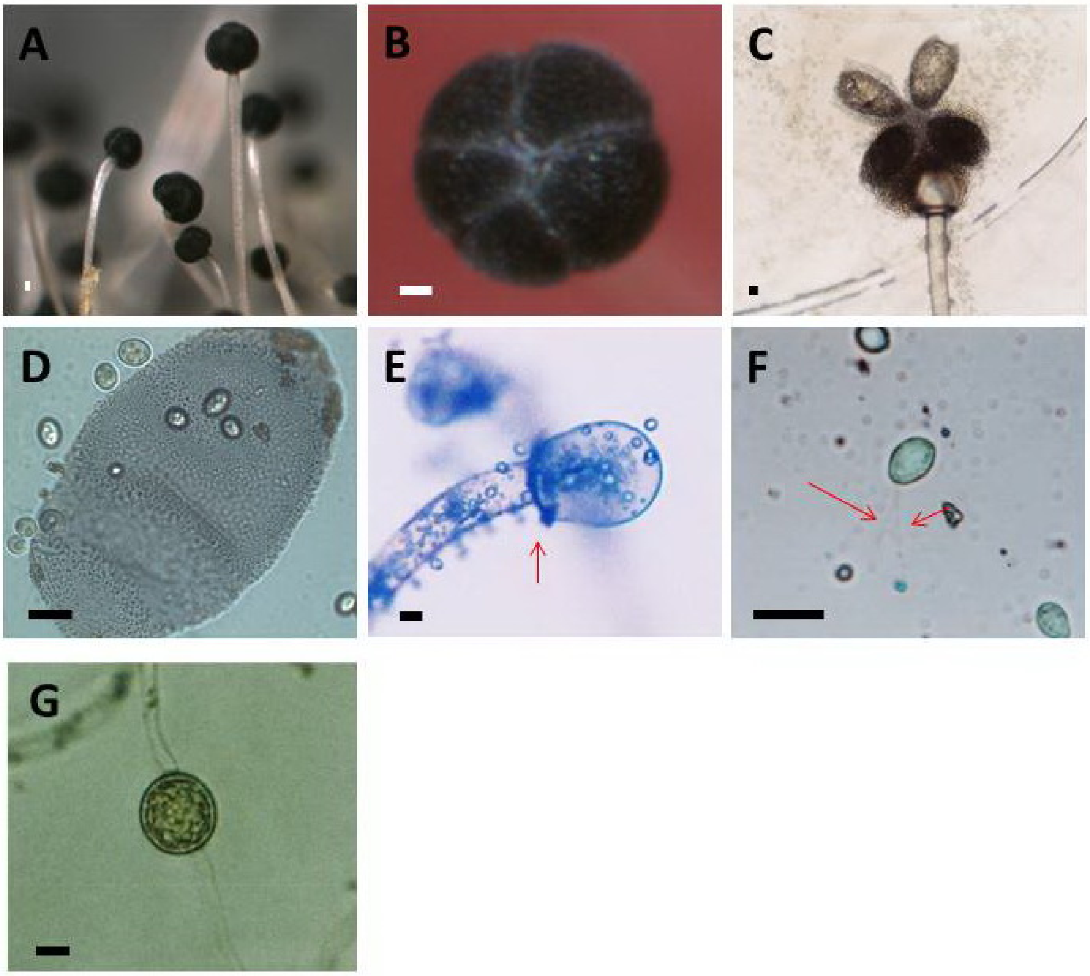

Fig. 2. Morphology of asexual reproduction structures of *Gibertella persicaria* var. *pitaya* produced on fruit tissues (A - B) and PDA (C - F). A: mature sporangia produced on curved (mostly) sporangiophores. B: persistent sporangial wall with longitudinal sutures. C: sporangial wall could separate into 2 - 5 pieces (in the case of this picture were 4 pieces). D: sporangial wall covered with crystalline spines. E: a columella with collar (arrow). F: a spore with two hyaline appendages (arrows), stained by rose bengal. G:. an intercalary chlamydospore produced by *G. persicaria* var. *pitaya* on MEA. Bar = 10 μm.

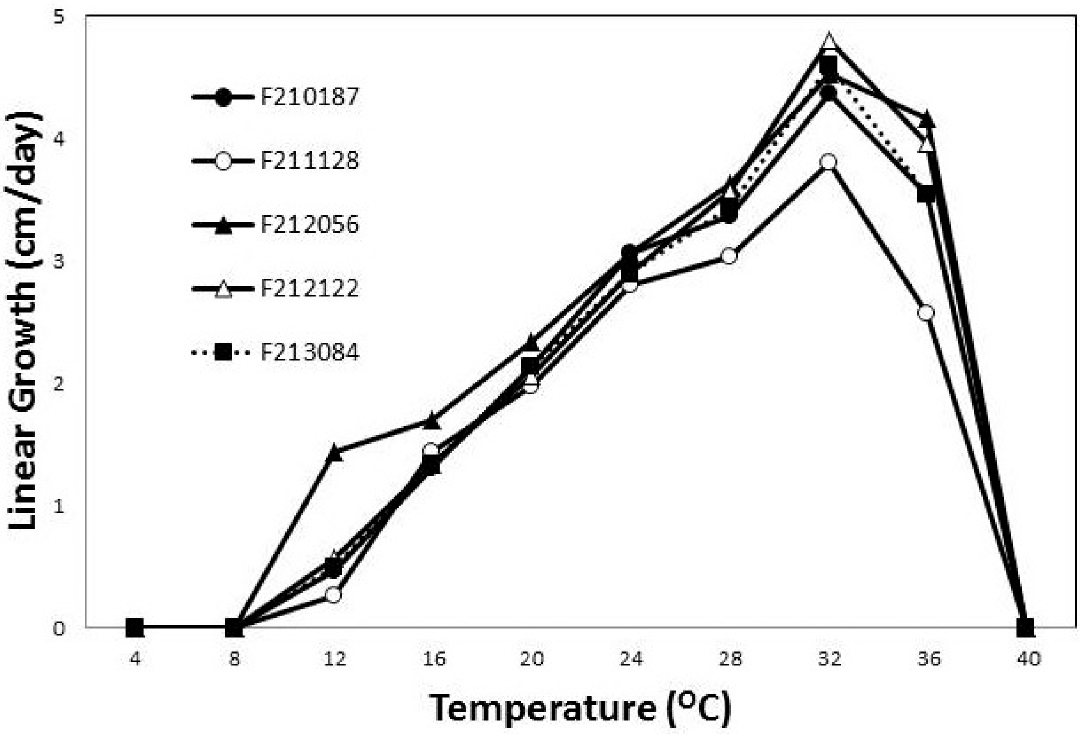

Fig. 3. Effect of temperature on mycelial growth of *Gibertella persicaria* var. *pitaya* isolates. Cultures were grown on PDA in petri dishes (diam. 9 cm) for 2 days. Linear growth rates were shown as average of 3 replicates of each isolates in a single experiment. This experiment was repeated for 3 times with similar results.